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Disorders of haemoglobin in China

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SUMMARY A large scale survey of haemoglobinopathies and thalassaemia has been carried out in China, involving 900 000 people in 28 provinces. It has resulted in the finding of many new variants and some interesting cases of thalassaemia, and in a study on the chemical structure of abnormal haemoglobins and DNA analysis of thalassaemia. We report here data on haemoglobin disorders in the Chinese, mainly the characterisation of the geographical distribution of haemoglobin variants, the analysis of globin genes of α, β, γ, or δβ thalassaemia, and the progress in prenatal diagnosis of α and β thalassaemia conducted in the authors' laboratory.

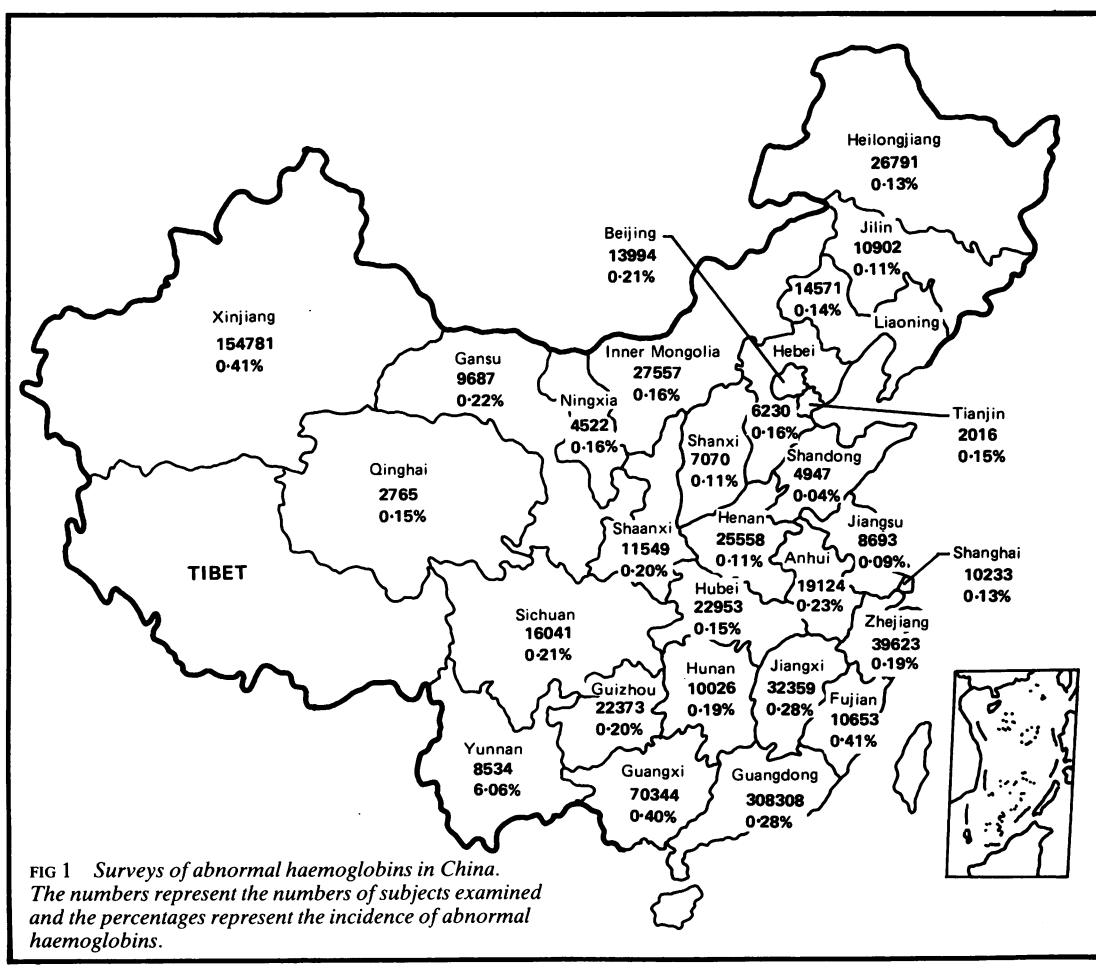

FIG 1 *Surveys of abnormal haemoglobins in China. The numbers represent the numbers of subjects examined and the percentages represent the incidence of abnormal haemoglobins.*

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The People's Republic of China is situated in the south-eastern part of the Eurasian continent and has a total land area of 9·6 million square kilometres. It is divided into 22 provinces, five autonomous regions (Inner Mongolia, Ningxia Hui, Guangxi Zhuang, Xinjiang Weiwuer, and Tibet), and three municipalities (Beijing, Shanghai, and Tianjin) directly under the central authorities (fig 1). China is a unified, multinational country (56 races), with a population of about one billion. In recent years a large scale survey of abnormal haemoglobins and thalassaemia was undertaken in China among 35 races, involving more than 900 000 people in 28 provinces (autonomous regions and municipalities).[1–3] The analysis of the chemical structure of haemoglobins and the study of globin genes of thalassaemia were conducted in various laboratories in China. In this paper we summarise the data on haemoglobin variants and thalassaemia and report some results of the analysis of globin genes in China.

Haemoglobin variants

Using electrophoretic techniques, blood haemolysates from 902 204 people were examined. A total of 2936 pedigrees with abnormal haemoglobins was found. The incidence of abnormal haemoglobins was 0·33% and the incidence in various regions in China are shown in table 1 and fig 1. The data show that Yunnan province has the highest incidence (6·06%) of Hb variants, where much haemoglobin E was found.

By fingerprinting or HPL chromatography, the chemical structure of 59 haemoglobin variants has been identified in about 700 families in China. Among them, 20 are new variants (table 2).

TABLE 1 *Surveys of abnormal haemoglobins conducted in China.*

Areas surveyed	*Total No of subjects examined*	*No of families with an abnormal Hb*	*Incidence of abnormal Hb (%)*
Yunnan	8534	517	6·06
Fujian	10 653	44	0·41
Xinjiang	154 781	634	0·41
Guangxi	70 344	280	0·40
Guangdong	308 308	858	0·28
Jiangxi	32 359	89	0·28
Anhui	19 124	43	0·23
Gansu	9687	21	0·22
Beijing	13 994	29	0·21
Guizhou	22 373	45	0·20
Shanxi	11 549	23	0·20
Zhejiang	39 623	76	0·19
Sichuan	16 041	30	0·19
Hunan	10 026	19	0·19
Hebei	6230	10	0·16
Inner Mongolia	27 557	43	0·16
Ningxia	4522	7	0·16
Hubei	22 953	35	0·15
Tianjin	2016	3	0·15
Qinghei	2765	4	0·15
Liaoning	14 571	20	0·14
Heilongjiang	26 791	35	0·13
Shanghai	10 233	13	0·13
Shaanxi	7070	8	0·11
Jilin	10 902	12	0·11
Henan	25 558	28	0·11
Jiangsu	8693	8	0·09
Shandong	4947	2	0·04
Total	902 204	2936	0·33

TABLE 2 *Haemoglobin variants found in China.*

	Residue	*Substitution*	*Name*
α chain variants	2(A2)	Leu→Arg	*Hb Chongqing
	11(A9)	Lys→Gln	*Hb Wuming-Wenchang
		Lys→Glu	Hb Anantharaj
	15(A13)	Gly→Arg	Hb Ottawa
	16(A14)	Lys→Asn	*Hb Beijing
		Lys→Glu	Hb I
		Lys→Met	*Hb Harbin
	18(A16)	Gly→Arg	Hb Handsworth
	19(AB1)	Ala→Glu	*Hb Tashikuergan
	26(B7)	Ala→Glu	*Hb Shenyang
	27(B8)	Glu→Lys	*Hb Shuanfeng
		Glu→Ala	*Hb Xuchang
	30(B11)	Glu→Gln	Hb G Chinese
	34(B15)	Leu→Arg	Hb Queen's
	42(C7)	Tyr→Asp	*Hb Huaxi
	48(CE6)	Leu→Arg	Hb Montgomery
	50(CE8)	His→Asp	Hb J Sardogna
	51(CE9)	Gly→Arg	Hb Russ
	54(E3)	Gln→Glu	Hb Mexico
	56(E5)	Lys→Thr	Hb Thailand
	64(E13)	Asp→Gly	*Hb Guangzhou
	68(E17)	Asn→Asp	Hb Ube-2
		Asn→Lys	Hb G Philadelphia
	74(EF3)	Asp→His	Hb G Taichung
	75(EF4)	Asp→Ala	*Hb Duan
	77(EF6)	Pro→Arg	*Hb Guizhou
	78(EF7)	Asn→Lys	Hb Stanleyville II
	87(F8)	His→Tyr	Hb M Iwata
		His→Arg	Hb Iwata
	116(GH4)	Glu→Lys	Hb O Indonesia
β chain variants			
	6(A3)	Glu→Val	Hb S
		Glu→Lys	Hb C
	6 or 7	Glu→O	Hb Leiden
	7(A4)	Glu→Lys	Hb Siriraj
		Glu→Gly	Hb G San Jose
	8(A5)	Lys→Gln	*Hb J Luhe
	10(A7)	Ala→Asp	Hb Ankara
	22(B4)	Glu→Gly	Hb G Taipei
		Glu→Ala	Hb G Coushatta
		Glu→Gln	Hb D Iran
	26(B8)	Glu→Lys	Hb E
	29(B11)	Gly→Asp	Hb Lufkin
	51(D2)	Pro→Arg	Hb Willamette
	56(D7)	Gly→Arg	Hb Hamadan
		Gly→Asp	Hb J Bangkok
	59(E3)	Lys→Asn	Hb J Lome
	64(E8)	Gly→Asp	Hb J Calabria
	78(EF2)	Leu→Arg	*Hb Quinhai
	80(EF4)	Asn→Lys	Hb G Szuhu
	113(G15)	Val→Glu	Hb New York
	120(GH3)	Lys→Ile	*Hb Jianghua
	121(GH4)	Glu→Gln	Hb D Punjab
	127(H5)	Gln→Glu	Hb Complutense
	131(H9)	Gln→Pro	*Hb Shanghai
	144(GH1)	Lys→Asn	Hb Andrew-Minneapolis
γ chain variants			
	22(B4)	Asp→Gly(GγI)	*Hb F Urumqi
	25(B7)	Gly→Arg(AγI)	*Hb F Xinjiang
	66(E10)	Lys→Arg(GγI)	*Hb F Shanghai
	73(E17)	Asp→His(AγI)	*Hb F Xin-Su

*New variants.

The data on the geographical distribution of Hb variants in China show that Hb E, New York, G Chinese, Q Thailand, and J Bangkok are mainly distributed over provinces of South China. These abnormal haemoglobins are also common in adjoining South-East Asia. In contrast, Hb D Punjab is principally found in the north of China. This variant is more common in India and South-West Asia, and it is considered that Hb D Punjab in China and India may have the same origin. It is possible that as early as in the Han dynasty (2000 years ago), the Hb D Punjab gene was introduced through the 'Silk Road' from India to China. In addition, a rare variant, Hb Queen's, was first found in Koreans. In China this variant is also found in the Korean nationality and the coastal areas close to Korea.

α thalassaemia

A total of 12 821 samples of cord blood from newborn babies was screened by electrophoresis in seven provinces (autonomous regions and municipalities) of China; 339 cases of α thalassaemia were found with raised levels of Hb Bart's. The incidence of this disease was calculated to be 2·64%. The incidence of α thalassaemia in the various areas of China is shown in table 3.[1]

The data show that the incidence of α thalassaemia in Guangxi Zhuang autonomous region bordering Vietnam is the highest (14·95%), where Hb H disease and Hb Bart's hydrops fetalis are serious problems. The high frequencies of α thalassaemia mutations have perhaps resulted from selective advantage of heterozygotes for these mutations over wild type homozygotes.

By restriction endonuclease mapping, we analysed the α globin genes of 60 unrelated Hb H patients and many of their parents. Three types of α thalassaemia were identified: the leftward (4·2 kb) deletion, the rightward (3·7 kb) deletion, and a non-deletion type. The results, listed in table 4 and fig 2, show that different types of Hb H disease are

TABLE 3 *The incidence of α and β thalassaemia in China.*

Areas surveyed	*Total No*	*No of cases*	*%*
α thalassaemia*			
Guangxi	301	45	14·95
Guangdong	4310	177	4·11
Jiangxi	769	20	2·60
Sichuan	4007	77	1·92
Zhejiang	1000	12	1·20
Xinjiang	859	4	0·47
Shanghai	1575	4	0·25
Total	12 821	339	2·64
β thalassaemia			
Guizhou	8655	191	2·21
Sichuan	7525	164	2·18
Guangxi	52 471	800	1·52
Guangdong	102 356	1110	1·08
Hunan	5178	19	0·37
Liaoning	346	1	0·29
Yunnan	370	1	0·27
Jiangxi	31 590	56	0·18
Fujian	1276	2	0·16
Hubei	21 603	19	0·09
Shanghai	12 017	8	0·07
Xinjiang	117 951	29	0·02
Total	361 338	2400	0·66

*Including α thalassaemia 2, α thalassaemia 1, Hb H disease, and Hb Bart's hydrops fetalis.

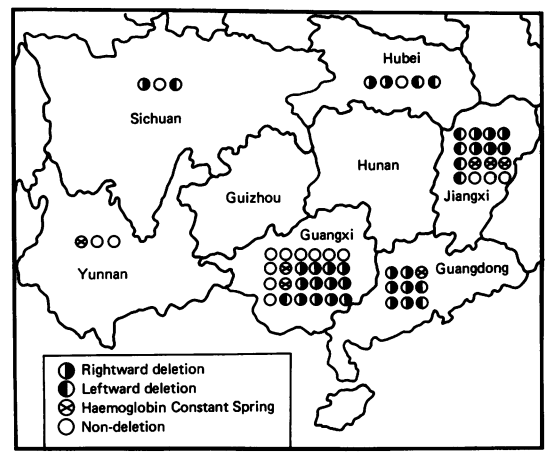


FIG 2 *Geographical distribution of various types of Hb H disease in South China.*

TABLE 4 *The distribution of α globin genes in Hb H disease among the Chinese.*

Province	*Case No*	*Non-deletion*		*Deletion*	
		$\alpha\alpha^{T}/--$	$\alpha\alpha^{CS}/--$	α−/−− *(−3·7 kb)*	−α/−− *(−4·2 kb)*
Guangxi	24	9	2	12	1
Guangdong	9	0	1	6	2
Jiangxi	16	3	3	6	4
Hubei	5	1	0	2	2
Yunnan	3	2	1	0	0
Sichuan	3	1	0	1	1
Total	60	16	7	27	10

distributed in different regions. The rightward deletion is mainly found in the Guangdong province, the leftward deletion is often found in the Jiangxi province, while the non-deletion type is mostly observed in the Quangxi region, where the main population is of the Zhuang nationality.[4]

For prenatal diagnosis of α thalassaemia, two methods, namely restriction endonuclease mapping and rapid DNA dot hybridisation, were used in our laboratory.[4] The technique of DNA dot hybridisation is based on the principle that the number of α globin genes in human cell DNA is proportional to the intensity of the autoradiogram. The DNA sample is applied directly onto the nitrocellulose membrane for hybridisation with labelled globin complementary DNA. The radioactive intensity of the DNA spot on the nitrocellulose filter identifies the number of α globin genes. This method has the advantage of simplicity, rapidity, and economy, and requires only 5 μg DNA. The whole process can be completed within 33 hours.[4] This technique was successfully applied in 13 pregnancies at risk of haemoglobin Bart's hydrops fetalis, in one at risk of haemoglobin H disease, and in two at risk of both disorders.

β thalassaemia

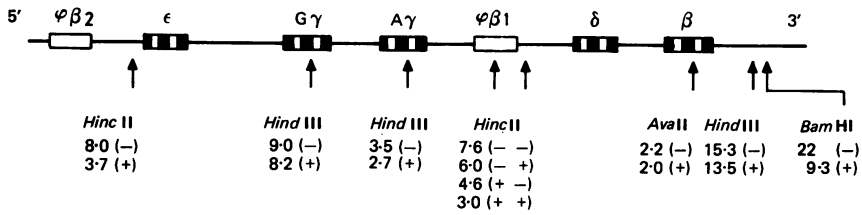
Using cellulose acetate electrophoresis for the quantification of Hb A_2 and an alkali denaturation procedure for the determination of Hb F, more than 360 000 people in China were screened for β thalassaemia and 2400 cases of the disease were found. The incidence of β thalassaemia was calculated as 0·66%. The regional incidence rates are shown in table 3.[1] As is shown in table 3, the incidence of β thalassaemia is higher in the south than in the north. This is similar to the geographical distribution of β thalassaemia generally in the world, in that the incidence is higher in the South-East Asian regions bordering the south of China and lower in the neighbouring countries to the north of China, such as the Soviet Union and the People's Republic of Mongolia.

A systematic study of DNA restriction fragment length polymorphisms (RFLPs) and haplotypes in the Chinese β globin gene cluster was conducted by the authors using seven DNA probes specific for 13 polymorphic sites. Table 5 shows the frequencies of these polymorphic sites in the Chinese, and compares the frequencies in different racial or ethnic groups. Haplotyes were determined at eight polymorphic sites and showed that 13 different types were associated with 50 chromosomes carrying the β thalassaemia (β^T) gene (fig 3). Among them 10 haplotypes have not been previously reported.[5]

To date six different mutations leading to β thalassaemia have been found in the Chinese population.[6 7] The six types are (a) A→G substitution at TATA Box −28 bp; (b) G→C substitution at IVS-1, position 5; (c) A→T substitution in codon 17; (d) four base pair deletion (CTTT) in codons 41 to 42; (e) nucleotide insertion of A between codons 71 to 72; and (f) C→T substitution at IVS-2, position 654. Using synthetic oligonucleotide (nonadecamers) probes, homologous to the six β^T mutations, as well as their normal counterparts, DNA from 10 β thalassaemia patients and their parents, living in Sichuang, Zhejiang, Guangxi, and Hunan of China, was analysed (table 6). The data listed in table 6 are in contrast to previous reports,[6 7] in which most of the mutant genes were caused by type E and F mutations and none by type A. This discrepancy could be due to the differences in the geographical locations of the Chinese patients in these studies. In addition, although previous reports showed a close association of haplotypes and specific mutations in an ethnic group,[7 8] our data showed that four types

TABLE 5 *Frequency of DNA polymorphic sites in the β globin gene cluster in different racial groups.*

RFLP	*Greeks*		*Italians*		*American Blacks*		*Indians*		*Chinese*	
	β^A	β^T	β^A	β^T	β^A	β^S	β^A	β^T	β^A	β^T
*Hinc*II 5′ε	0·46	0·85	0·76	0·54	0·10	0·02	0·78	0·75	0·75	0·66
*Hind*III Gγ	0·52	0·14	0·26	0·48	0·41	0·35	0·30	0·26	0·19	0·08
*Hind*III Aγ	0·30	0·07	0·06	0·37	0·16	0·05	0·06	0·09	0·28	0·00
*Pvu*II φβ$_1$	0·27	0·16					0·62	0·04	0·38	0·20
*Hinc*II φβ$_1$	0·17	0·07	0·20	0·11	0·15	0·04	0·17	0·10	0·07	0·08
*Hinc*II 3′φβ$_1$	0·48	0·12	0·28	0·31	0·76	0·81	0·27	0·17	0·15	0·08
*Hinf*I 5′β	0·97	0·92	0·95	0·92	0·70	0·10	1·00	0·86	0·91	0·65
*Rsa*I 5′β									0·51	0·45
*Hgi*AI 5′β	0·80	0·90	0·86	0·73	0·96	0·96	0·82	0·38	0·47	0·35
*Ava*II β	0·80	0·90	0·86	0·73	0·96	0·96	0·78	0·38	0·49	0·60
*Hpa*I 3′β	1·00	1·00	1·00	1·00	0·93	0·35	1·00	1·00	0·89	0·95
*Hind*III 3′β	0·72				0·63		0·56		0·33	0·10
*Bam*HI 3′β	0·70	0·78	0·74	0·82	0·90	1·00	0·82	0·84	0·62	0·88



Types	Haplotypes							No of chromosomes	Frequency
I	+	–	–	– –	+	–	+	18	36%
II	+	–	–	– –	–	–	+	10	20%
*III	–	–	–	– –	–	–	+	6	12%
*IV	–	–	–	– –	+	–	+	4	8%
V	+	–	–	– –	+	+	–	3	6%
*VI	–	–	–	– –	–	–	–	2	4%
*VII	+	+	–	– –	–	+	–	1	2%
*VIII	–	–	–	+ +	+	–	+	1	2%
*IX	–	–	–	+ –	+	–	+	1	2%
*X	+	+	–	– –	+	–	+	1	2%
*XI	–	–	–	– +	–	–	+	1	2%
*XII	–	+	–	+ +	+	+	+	1	2%
*XIII	–	+	–	+ +	+	–	+	1	2%
Total								50	100%

*New haplotypes

FIG 3 *Haplotype of β^T chromosomes in the Chinese. Each polymorphic site is shown by an arrow. The number under the restriction endonuclease is the size of DNA fragments (kb). + indicates presence of cleavage at a particular site; – indicates absence of cleavage at a particular site.*

TABLE 6 *RSP haplotypes of types of β thalassaemia gene.*

No of families	*Origin*	*Restriction site polymorphism haplotype*	*Types of β thalassaemia mutations*					
			TATA Box-28 A→G	*IVS-1 No 5 G→C*	*Codon 17 A→T*	*Codons 41–42 –4 bp*	*Codons 71–72 +A*	*IVS-2 No 654 C→T*
1	Shanghai	Paternal –+–+++++						+
		Maternal –+–+++–+		+				
2	Zhejiang	Paternal +––––––+					+	
		Maternal +––––+–+				+		
3	Sichuang	Paternal ++–––+–+			+			
		Maternal +––––––+						+
4	Hunan	Paternal +––––––+					+	
		Maternal +––––+–+				+		
5	Zhejiang	Paternal +––––+–+				+		
		Maternal +––––––+					+	
6	Guangxi	Paternal +––––+–+			+			
		Maternal –––––––+				+		
7	Sichuang	Paternal ––––+––+	+					
		Maternal +––––+–+					+	
8	Sichuang	Paternal +––––+–+						+
		Maternal +––––+–+				+		
9	Guangxi	Paternal +––––+–+				+		
		Maternal +––––+–+				+		
10	Hunan	Paternal ––––––+		+				
		Maternal –––––––+				+		
No of chromosomes		20	1	2	2	8	4	3
Percentage		100	5	10	10	40	20	15

of mutations (C,D,E,F) were found in a haplotype +––––+–+, the commonest type of Chinese β^T mutation.[9] A plausible explanation is that the multiple mutations found in this haplotype may have arisen by gene conversion.

Prenatal diagnosis of β thalassaemia was performed in Shanghai Children's Hospital by linkage analysis of RFLPs, as well as by synthetic oligonucleotide hybridisation.[10] Oligonucleotide hybridisation provides a very effective method for direct

detection of the mutation and for prenatal diagnosis of β thalassaemia. We used the synthetic oligomer probes specific for the normal and mutant sequence of codons 41 to 42 (−4 bp) to diagnose two fetuses at risk of β thalassaemia, which cannot be diagnosed by linkage analysis of RFLPs, identifying one fetus as having β thalassaemia trait and the other as having β thalassaemia major.

γ thalassaemia, δβ thalassaemia, and HPFH

During an analysis of the γ globin chain composition of over 1100 Chinese newborn babies by HPLC, we found 25 babies who were heterozygotes for γ thalassaemia, while one baby was a homozygote with Hb F consisting of α chains and Aγ chains only. Gene mapping of the DNA from this baby and his parents identified the baby as a homozygote for −GγAγ thalassaemia which is caused by a deletion of about 5 kb due to an unequal crossing over between the −Gγ and −Aγ genes. The frequency of the −GγAγ gene among babies from the Shanghai area may be as high as 0·012%.[11]

In addition, a few cases of HPFH and δβ thalassaemia were found in various provinces throughout the south and north of China. DNA from the three families with δβ thalassaemia or HPFH was analysed by extensive restriction endonuclease mapping with a battery of restriction enzymes and probes.[12] The first concerns a Gγ(Aγδβ)° thalassaemia found in a relatively large family from Canton. This type of thalassaemia was characterised by a large deletion originating 3′ to the Gγ globin gene and extending beyond sequences recognised by the pRK28 probe. The abnormality is different from similar conditions found in families from other countries. We named it the Cantonese type after the area where members of the family are living. The second was a most unusual GγAγ(δβ)$^+$ HPFH condition present in a large family from mid China; this anomaly closely resembled the different HPFH types found in Blacks, except that no deletion was present. The third was a Aγ(δβ)$^+$ HPFH type which excludes the presence of a deletion of any signficant size and resembles those seen in Greece and England.

Conclusion

In summary, the Chinese are the most populous ethnic group in the world. Friendly contacts between the Chinese people and the people of other countries began as early as 2000 years ago. Thus, the study of haemoglobin disorders in the Chinese will contribute considerably to the understanding of the historical, racial, migrational, and genetic relationships between the Chinese and other nations of the world. Previous studies of haemoglobin disorders conducted in China have revealed many different haemoglobin abnormalities in the Chinese population; further study on the molecular defects of Hb disorders will reveal the molecular mechanisms of abnormal globin gene expression and the results of these studies may be applied to the diagnosis and therapy of haemoglobin disorders and also to other genetic diseases.

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References

1. China Hemoglobinopathy Cooperative Study Group. A survey of hemoglobinopathy in 600,000 people of 20 provinces, cities and autonomous regions of China. *Natl Med J China* 1983;**63**:382–5.
2. Institute of Hematology of the Chinese Academy of Medical Sciences. Epidemiologic analysis of abnormal hemoglobins in China. *Natl Med J China* 1986;**66**:462–4.
3. Ren Bang-zhe. Study on abnormal hemoglobin in China. *Chem Life* 1986;**6**:2–5.
4. Zeng YT, Huang SZ. α-globin gene organization and prenatal diagnosis of α-thalassaemia in Chinese. *Lancet* 1985;**i**:304–7.
5. Zeng YT, Huang SZ, Zhou XD, *et al.* A study on restriction endonuclease site polymorphism and haplotype in the β-globin gene cluster in Chinese. *Acta Genet Sinica* 1986;**13**:317–22.
6. Antonarakis SE, Kazazian HH Jr, Orkin SH. DNA polymorphism and molecular pathology of the human globin gene cluster. *Hum Genet* 1985;**69**:1–14.
7. Cheng TC, Orkin SH, Antonarakis SE, *et al.* β-thalassemia in Chinese: use of in vivo RNA analysis and oligonucleotide hybridization in systematic characterization of molecular defects. *Proc Natl Acad Sci USA* 1984;**81**:2821–5.
8. Orkin SH, Kazazian HH Jr, Antonarakis SE, *et al.* Linkage of β-thalassemia mutations and β-globin gene polymorphisms with DNA polymorphisms in human β-globin gene cluster. *Nature* 1982;**296**:627–31.
9. Huang SZ, Law ML, Zeng YT, Zhang J, Kan YW. β-thalassemia mutations in Chinese subjects identified by synthetic oligonucleotides. Abstract (M IV.31) *7th International Congress of Human Genetics, Berlin, September, 1986.*
10. Zeng YT, Huang SZ, Sheng M, *et al.* Prenatal diagnosis of β-thalassemia. *Shanghai Med J* 1985;**8**:559–64.
11. Zeng YT, Huang SZ, Nakatsuji T, Huisman THJ. -GγAγ-thalassemia and γ-chain variants in Chinese newborn babies. *Am J Hematol* 1985;**18**:235–42.
12. Zeng YT, Huang SZ, Chen B, *et al.* Hereditary persistence of fetal hemoglobin or (δβ)°-thalassemia: three types observed in South Chinese family. *Blood* 1985;**66**:1430–4.

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